ANALYSIS OF INTRA-CHROMOSOMAL DELETIONS IN YEAST

Research Thesis

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by

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ABSTRACT

Deoxyribose Nucleic Acid (DNA) Double Strand Breaks (DSBs) are the most dangerous form of chromosome damage because they result in a severed chromosome. If the chromosome doesn't properly repair itself it could lead to major chromosomal abnormalities such as deletions, translocations, inversions, duplications and other kinds of copy number variations; all characteristics of cancer. An accurate DNA damage response pathway is imperative for repair of DNA double strand breaks. Repair may occur by homologous recombination of which many different sub-pathways have been identified. Some pathways are conservative meaning that the chromosome sequences are preserved, and others are non-conservative leading to some alteration of DNA sequence. The project focused on designing an *in vivo* genetic assay to study non-conservative intra-chromosomal deletions at regions of non-tandem direct repeats in *Schizosaccharomyces pombe*. This assay can be used to study both spontaneous breaks arising during DNA replication and induced double strand breaks created with the *S. cerevisiae HO* homothallic endonuclease. Preliminary genetic characterization of this assay shows that spontaneous breaks require *rad52*$^{+}$ but not *rad51*$^{+}$ while induced breaks require both genes. This suggests that the two types of breaks have distinct genetic requirements. This assay will be useful in the field of DNA damage repair for studying mechanisms off intra-chromosomal deletions. This assay was used to study the function of two chromatin remodeling genes: Mst1 (human TIP60) and Skb1 (human PRMT5). Mst1 is a histone acetyltransferase which functions to remove histones form the DNA double strand break and Skb1 is its regulator. It was revealed that both Mst1 and Skb1 promote conservative repair of DNA double strand breaks.

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INTRODUCTION

DNA is comprised of four different nucleic acid bases, adenine, guanine, thymine, and cytosine. Adenine and guanine are purines that bind to the pyrimidine bases thymine and cytosine respectively. DNA stores all our genetic information and passes it along to the next generation of cells by replicating itself. Replication of DNA is said to be semiconservative because the two strands of DNA are separated from one another to form template strands, the template is read, and new nucleic acids are inserted [1]. The single stranded DNA (ssDNA) is re-annealed together to form two separate helices with half old DNA and half new DNA in each. Due to the semiconservative nature of DNA, mutations such as insertions, deletions, or substitutions would have to go through two rounds of replication unnoticed before they become permanent or fixed [2]. The repair machinery may catch and repair mistakes during this time. Even though these repair mechanisms are extremely reliable at preventing mutation accumulation, some errors still escape. With time, these errors may accumulate to sufficiently change the genetic makeup to cause cancer.

The chromosomes of eukaryotes, such as humans, are bound by specific proteins known as histones. The function of histones is to package the genome into the nucleus and regulate gene expression. Eukaryotes have another problem associated with replication, they have to remove every histone from the DNA, replace half the histones with new ones, and reposition histones back on to the DNA. This is important because the position of histones on the chromosome determines the level of gene expression. Generally, histone density regulates how frequently genes are transcribed; the less histones there are the higher the transcription level of the gene [3]. As genomes increased in size, the repair mechanisms and histone remodeling machinery had to evolve concomitantly to prevent errors during genome duplication. In addition to changes in gene expression, if the histones are not properly removed or repositioned onto chromosomes after replication, the replication machinery may stall, collapse and cause various forms of DNA damage including DNA double strand breaks [4].

Causes and types of DNA damage. DNA damage can be caused by exogenous and endogenous factors which are both main sources of DSBs [5]. Exogenous DNA damage develops from external factors while endogenous DNA damage is caused by internal factors. Surprisingly, the majority of DSBs occur endogenously because of DNA replication errors. Whether the damage is endogenous or exogenous, it is repaired by the same type of repair mechanisms [2].

Mutations. Mutations come in many different forms that vary in severity [2]. The point mutation is a mutation that results in the change of only one or very few nucleotides in a gene sequence. The least consequential point mutation is a *silent mutation* in which the mutation doesn't change the amino acid outcome. The genetic code is degenerate, meaning that there are events where different codons specify for the same amino acid. Each of the 64 codons is only used for one of the 23 amino acid or stop code, no two amino acids have the same code. *Missense mutations* are point mutations that do change the amino acid for which it is coded. These mutations can pose a real threat to the phenotypic expression of proteins depending on how different the amino acids are and what it is used for. This could be anything from an enzyme active site that can no longer accept its substrate to a just slightly misshaped protein which could cause a partial or complete loss of function. An *insertion or deletion (InDel)* event is where a single base gets either put into or removed from the DNA. This changes not only that one codon, but all of the codons in that sequence causing a "reading frame" shift. The reading frame is a set of three nucleic bases that are grouped together and translated into one amino acid. This large of a mutation event is capable of knocking out a whole gene's function and possibly kills the organism. An equally, if not worse mutation event, is

the nonsense mutation. A *nonsense mutation* is a special kind of point mutation where one of the three stop codons is prematurely coded for causing the translation of the protein to stop abruptly and prematurely. This means that the gene will not be transcribed all the way to the wild type specifications but will be truncated. The severity of the loss and point at which it stopped could vary in how bad the outcome is, but once again it could cause the organism to lose function of an enzyme.

Chromosome breaks. Chromosome breaks pose a more serious form of DNA damage because they can cause loss of entire chromosome regions if repaired improperly. The chromosome breaks fall into two categories, single strand breaks and boule strand breaks. A *single strand break* results from breaking the covalent bonds of the sugar backbone of one of the DNA strands while keeping the other strand intact [6]. These breaks may be both induced and occur spontaneously. Repair of single strand breaks is generally thought as error-proof because an intact template exists (the non-broken strand) that provides the missing information. These mechanisms are not the focus of this thesis.

Improper repair of single strand breaks may result in the most dangerous form of DNA damage, the *double strand break* (DSB) which is breaking of both DNA strands [5]. This break is more serious because information on both strands is lost. DSBs can be induced by various exogenous factors but a vast majority of them arise during DNA replication. For example, replication forks may run into single strand

breaks that were not repaired and convert these breaks to DSBS. This causes the replication forks to stall and collapse. The only way to restart replication is with the assistance of the DNA damage repair mechanisms. These mechanisms are known as homologous recombination (HR). Exogenous factors can cause DSBs, but are less frequent and caused by radiation, high energy irradiation, or carcinogens. Ultraviolet radiation can cause thymine dimers that are normally taken care of by photoreactivation that cleaves out the dimer with photolyase enzymes, but if they are not taken resolved they can cause a DSB.

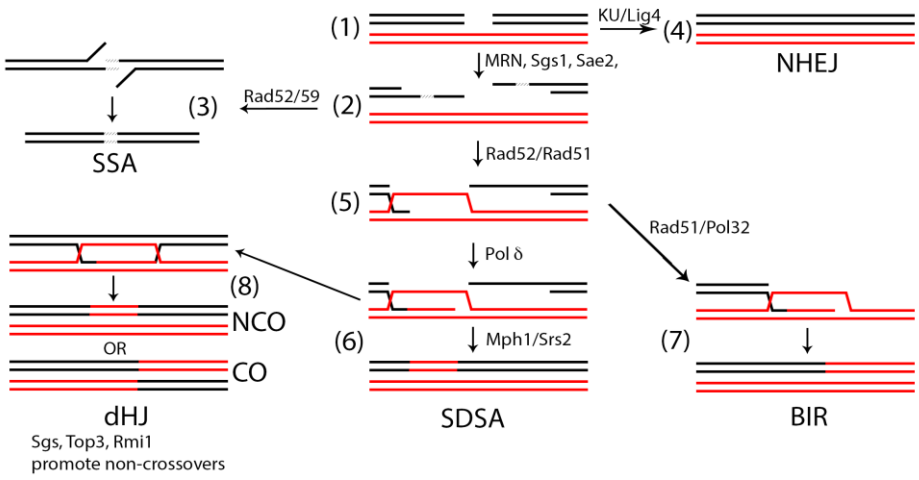

Figure 1. Pathways of repair of DNA double strand breaks. A diploid cell with two homologous chromosomes, black and red, sustains a double strand break (DSB) in the black chromosome (1). The DSB is first resected to expose ssDNA required for invasion of donor regions (2). If direct repeats (shaded areas) exist on the same chromosome, the break may be repaired by single strand annealing (SSA) (3). If repair fails, the chromosome may be lost (4). When homology is found elsewhere or on the other homologue (red), the broken ends may invade this region (the donor sequence) (5). In synthesis-dependent strand annealing (SDSA) (6) the invading strand may copy a small region then release and re-anneal. In break-induced replication (BIR) (7) the invading strand may copy to the end of the red chromosome. In this case the right portion of the broken black chromosome is lost. Occasionally a more complex double Holiday Junction (dJH) may be established (8), the resolution of which can result in crossovers (CO) or non-crossovers (NCO). Note that some of these repair outcomes may lead to loss of heterozygosity meaning that the black sequence has been converted to red. If the red sequence contains a recessive non-functional allele, some of these outcomes will convert the functional black allele to the non-functional red allele resulting in complete inactivation of the gene. Some of the genetic requirements for each pathway are indicated.

Homologous recombination mechanisms of DSB repair. DNA DSBs are much harder to repair because they lack their original template strand and are completely separated from the other half of the chromosome (**Fig. 1**). Because eukaryotic organisms such as humans are diploid (two copies of each chromosome) breaks often occur on only one of the chromosomes and repair may be accomplished by copying the missing region from the intact chromosome. A break usually creates two blunted chromosome ends (**Fig.1(1)**). In order for repair to occur the blunted DNA ends have to be resected (**Fig.1(2)**) to generate small regions of single stranded DNA. Two forms of non-conservative repair are Single strand annealing (SSA) and Non-Homologous End Joining (NHEJ). SSA only occurs if there are repetitive sequences of chromosomes on either side of the break ((**Fig.1(3)** shaded areas) [7]. In this case resection occurs past the repetitive elements then the unique sequences snap on each other and re-anneal the chromosome. The two flaps are then removed. Note that SSA leads to a deletion of the intervening sequence between the direct repeats and is therefore a nonconservative repair pathway.

Repair of DSBs can also proceed through Non-Homologous End Rejoining (NHEJ) (**Fig.1(4)**) which accounts for 70% of repair during G_2 phase of the cell cycle in humans. The remaining 30% are repaired through other "error-proof" HR pathways (**Fig.1(5-8)**) [8]. We use "error-proof" here cautiously because although it has been traditionally described as such, as we shall see, even these pathways may lead to some errors. In lower eukaryotes such as the yeast models, 90% of break repair is accomplished by error-proof HR and the remaining 10% by NHEJ [5]. In NHEJ, the sticky ends of the chromosome just reattach to one another quickly by using a special set of enzymes (**Fig.1(4)**). This is known to be error prone repair because some low level of resection still occurs which may lead to loss of information [9].

The error proof HR mechanisms of repair use an intact chromosome (black) as a template from which to copy the missing sequence. The downside to this method is that it exposes the naked single stranded DNA longer as it invades the homologous chromosome. Single stranded DNA is vulnerable to being targeted for degradation because it looks like the parasitic genome of a virus, so time spent in this stage is dangerous. Error proof HR can be further divided into several other pathways that can often lead to major sequence rearrangements or deletions. In Synthesis Dependent Strand Annealing (SDSA) (**Fig.1(5-6)**) the single stranded DNA may invade a homologous sequence somewhere in the intact chromosome (black). DNA polymerase then copies a short track of this sequence and re-anneals the end to the other fragment of the black chromosome. Note that because the two homologues chromosomes (black and red) are similar but not completely identical some of the black sequence has been changed to red. Often a gene would have two alleles (two versions of the same gene, black and red) one functional and one nonfunctional (heterozygote). One copy of the functional allele is sufficient to promote normal function. In this case if the black allele happened to be the functional one both homologous chromosomes have changed to a non-functional version of the gene. Therefore, this form of repair may lead to some errors and not be completely error-proof. In genetics we call this form of repair which changes one allele to another gene conversion.

Sometimes one piece of the broken chromosome is completely lost, and repair may occur by a process known as break-induced replication (BIR) (**Fig.1(7)**) [10]. In our example, if we assume that the right segment of the black chromosome is lost, the left segment may invade the red chromosome and copy all the way to the end of the chromosome. Note that BIR causes gene conversion of large chromosomal regions and therefore it could be thought of as more mutagenic than SDSA.

Finally, a rare form of repair known as the Double Holliday Junctions (dHj) which is normally restricted to meiosis may also occur ((**Fig.1(8)**) [11]. This form of repair is beyond the scope of this thesis, but I only want to mention that it is accomplished by a more complex entanglement of the red and black chromosomes (the two homologues). Resolution of this entanglement may cause either gene conversion (NCO) or a translocation (CO). Translocations are desired in meiosis where shuffling of genes between homologous chromosomes is important for genetic variation but not in mitosis (normal cell division) where it can cause alteration of gene expression leading to cancer. Therefore, this form of repair could be mutagenic.

Genetic requirements for the different forms of repair.

Resection. Following generation of a DSB the MRN complex is the first to appear and is sometimes referred to as the break sensor [9]. MRN is a complex of three proteins (Mre11, Rad50 and Nbs1). Remarkably MRN has two functions: 1) holds the two broken ends together and may cap them to prevent further degradation and 2) assists with localized resection. MRN plays the initial role in in repair by also signaling the repair machinery. Mre11 has both 3' to 5' endo and exonuclease activity to re-sect the ssDNA. If long range resection is required, MRN recruits other more processive exonucleases such as Exo1

and Sae2 as well as several helicases such as Sgs1 [12]. Slow growth suppressor 1 (Sgs1) is a DNA helicase protein that is central to most homologues recombination and pushes repair towards non-crossover HR. Sae2, antagonizes MRN complex's function. It appears that too much recombination may be detrimental, so the cell has in place mechanisms to balance the level of recombination.

Ku mediated Non-Homologous End Joining (NHEJ). If the break is to be repaired by NHEJ, Ku may also interact to prevent further degradation. In fact, it appears that MRN competes with Ku for the broken ends. Ku biases repair towards NHEJ while MRN biases repair towards HR. The interplay between Ku and MRN has been well established and it will also be documented in this study. As mentioned before this is an error prone method of repair that goes through little to no proof reading or use of homology. The two broken ends are quickly ligated together and often have small deletions or insertions which can cause genetic change. Ku70 is required for NHEJ because it binds to the terminal ends of the breaks with high affinity, protects the ends from being degraded, and recruits the other NHEJ machinery. Ku is assisted in accomplishing NHEJ by several other proteins including Lig4, a ligase that connects the two broken ends together [13]. An important advantage to going through NHEJ is that it is able to happen at any point in the cell cycle and doesn't need access to its homolog whereas HR is restricted to S-phase.

Rad52/Rad59 Mediated Single Strand annealing (SSA). If repair is to proceed via HR, immediately after resection Replication Protein A (RPA) binds to one of the single stranded sections of DNA while exonuclease 1(Exo1) chews away at the other until it is far enough back to have a primer for polymerase [9]. The function of RPA is to protect the single stranded DNA as single stranded DNA is immediately degraded in eukaryotic systems. Once the DNA is resected, it is possible to search for homologies and either invade the homologous chromosome next to it or find homology within itself. **Figure 1** shows a single strand annealing (SSA) pathway involving a black chromosome that contains non-tandem direct repeat sequences within itself. The ssDNA degrades until homology is reached and the complimentary non-tandem direct repeats then anneal to one another. The flaps are then cleaved away and degraded by the Rad1-Rad10 endonuclease (XPF-ERCC1 in mammals) with assistance from Msh2-Msh3 mismatch repair proteins and Slx4 and Saw1 scaffolding proteins This process does not rely on the invasion of a homologous chromosome so Rad51 is not required (see next section). Remarkably Rad52 is still essential but is assisted by a different protein (Rad59). SSA is a deletion pathway leading to loss of some of the chromosome sequences.

Rad51 mediates HR. Rad52 is required for all events following resection, that is, those that do not proceed through Ku including SSA as we have seen above. This protein is conserved in all eukaryotes [14]. However, it appears that at the Rad52 junction a choice is made between Rad59 mediated repair and Rad51 mediated repair. In HR, the function of Rad52 is to remove RPA and load Rad51 onto the DNA. It is Rad51 which initiates the search for homology on a different chromosome and invasion. RPA still plays a role in stabilizing the D-loop of the template strand during SDSA (**Fig.1(5)**). Rad51 is assisted in this homology search by several other proteins including Rad455/Rad57 [5, 15]. Once homology has been found another protein, Rad54 then removes Rad51, which opens up space for DNA polymerase to synthesize DNA and repair the chromosome [16]. Polδ, a DNA polymerase will replicate the areas of the genome that are lined up with their homology. Resolution of entangled intermediates is facilitated by several helicases including Mph1 and Srs2. Both BIR and dHJ require the Rad52/Rad51 genetic mechanism.

Types of double strand breaks. Most DSBs are modeled as two-ended (**Fig.1, Fig.2A**). However, most breaks that occur during DNA replication are one ended (**Fig. 2B**). These forms of breaks are repaired mainly by Break Induced Replication (BIR) after a nick in the template strand which causes the replication fork to collapse forms a double strand break. The chromosome is broken without another half to attach

to, so it has no other option besides invading the homologous chromosome to repair itself. BIR also relies on Rad51 and resection mechanisms. However, during BIR, a replication fork is formed and Pol32 is able to replicate the missing sequence all the way to the end of the chromosome. By using this process, the cell is able to replicate a full chromosome but loses all of its heterozygosity over the replicated region.

Cancer. Cancer is categorized as the accumulation of mutations that cause chromosomal abnormalities. This is strange though because most of the mutations that humans build up in their life time happen during the "protected" stage of their life. This protected stage is when they are less likely to have access to exogenous sources like alcohol and cigarettes because they are young [18]. Research has shown that the high mutation rate is due to endogenous factors such as replication errors as they are growing rapidly. Yet few people get cancer in their 20's. Most of the cancer occurrences in humans happen around the age of 70, so there must be some other explanation for this mismatched timing [19].

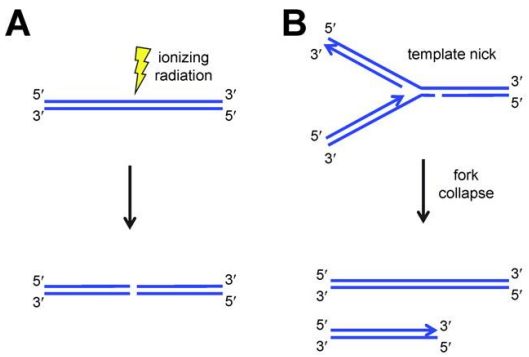

Figure 2. Types of DNA double strand breaks. A. If DSBs are produced by exogenous agents such as ionizing radiation, they are usually two-ended and they are repaired by mechanisms shown in **Figure 1**. **B**. DSBs arising during DNA replication are usually one-ended and occur as a result of replication forks running into unrepaired single strand breaks. Note that this produces a shorter chromosome as the ssDNA break is converted to the dsDNA break. These replication breaks are primarily repaired through BIR and rarely through any other mechanisms shown in **Fig.1.** Adapted from Dillingham and Kowalczykowski (microbiology and molecular biology reviews, 2008) [17].

The role of apoptosis and DNA damage checkpoints. When the cell encounters DNA it must either repair the damage completely or undergo apoptosis, programed cell suicide, in order to not become cancerous. The discussion of apoptosis has been excluded in this introduction because it is beyond the scope of this thesis, but it is important to note that a vast majority of cells that accumulate DNA damage will die [20]. Another exclusion was a discussion of the DNA damage checkpoints that assist with repair. The cell has to be able to arrest the cell cycle and keep the cell from dividing rapidly until the damage can be repaired [21].

Inactivation of cell cycle regulators. For a cell to become cancerous it must sustain mutations in special genes as cell cycle regulators. Most cancer genes are characterized by about 2,000-10,000 mutations, but only a few of these are transforming: causing a cell to become carcinogenic [19]. There are two major types of cell cycle regulators that must be inactivated. *Tumor suppressor genes* are genes that put the brakes on the cell cycle (checkpoint genes). So long as the cell is arrested at a check point it can't divide rapidly. If tumor suppressor genes are knocked out then the cell cycle can replicate unchecked, but still at a fairly slow rate [21]. *The proto-oncogenes* control cell growth by stimulating division. A mutation in a proto-oncogene will convert it to an oncogene (a hyperactive stimulator). This oncogene speeds up the cell cycle by being under constant stimulation even when no signal is given to divide. Once out of control the tumor can set up its support system and parasitize its host. One example of a well-studied proto-oncogene is Ras which is mutated in nearly 30% of the cancers [22, 23].

Chromatin remodeling and DNA damage repair. Chromatin remodeling to prepare the broken ends for recombination and proper re-assembly of histones following DSB repair is also important. Several

histone remodeling complexes have been identified that have roles in DSB repair [24-26]. The role of two chromatin remodelers, Mst1 (human TIP60) and Skb1 (human PRMT5) (**Fig. 3**) were researched.

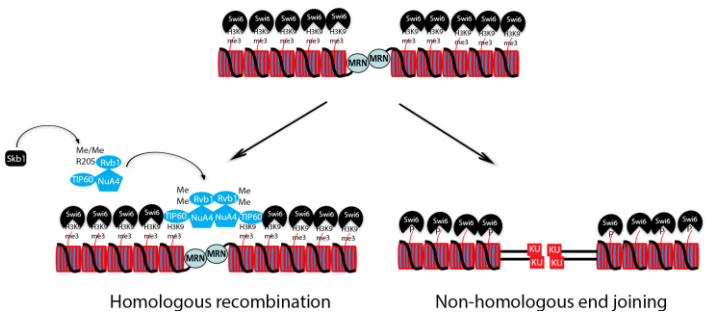

Figure 3. Model for the role of TIP60 and Skb1 in DNA damage repair. In fission yeast the break is still initially recognized by MRN. Methylated histones H3 are bound by the Swi6 protein. If the choice of repair is through homologous recombination the arginine methyltransferase Skb1 methylates the Rvb1 subunit of the NuA4 complex promoting its association with some of the H3K9me3 marks that have become available due to the displacement of Swi6. Non-homologous end joining is rare but it will lead to the recruitment of the KU complex. TIP60-NuA4 is not required for this.

TIP60. TIP60 belongs to a family of proteins with members in virtually all species studied that are characterized by the presence of a highly conserved MYST domain [27]. In *Schizosaccharomyces pombe (Sp)*, Mst1 is an essential histone acetyltransferase [28-30] functionally homologous with human TIP60. Esa1 is the catalytic subunit of a NuA4 complex that has been implicated in DSB repair [31]. In *Sc,* acetylation of histone H4 by Esa1 is required for efficient repair of a DSB [32]. Mst1 has been initially reported to be an essential gene that is more than 70% similar to Esa1 and TIP60 [33] making the relationship between Mst1 and human leukemia associated mutants very strong. A *mst1*^{ts} temperature sensitive allele is sensitive to numerous DNA damaging agents and to hydroxyurea, a drug that stalls replication forks [30]. In addition, in *Sp* Mst1 also contributes to establishment of the centromere as well as chromatin remodeling during transcription which appears to be its essential function [30, 34].

PRMT5. Skb1 (human PRMT5) is non-essential arginine methyltransferase initially identified in yeast to function in establishing cell morphology and polarity [35]. The gene has been shown in humans to have pleiotropic functions including in development and cancer [36]. In addition to its cell polarity targets, Skb1 methylate's several other targets including histones H2A, H4 and H3 in humans and H4R3 in plants.

TIP60 and PRMT5 role in DNA damage repair. The role of these proteins appears to be biasing repair towards conservative HR (**Fig.3**). The choice between repair by NHEJ and HR appears to be determined by the stage of the cell cycle, the degree of end resection, as well as through a competition for access of the break between NHEJ factors and HR factors [37]. MRN, the break sensor, interacts with the break first and may bias repair towards HR or NHEJ. The choice appears to be determined by the level of histone removal which tracks directly with resection: the more histone removal the longer the resection. The exact mechanism is not well understood. As mentioned earlier, in yeast, some evidence suggests that the NHEJ proteins Ku70 and Ku80 can load early and may even compete with MRN and resection proteins [38, 39].

TIP60 is required for acetylation of histone H4 at Lysine K16 (H4K16). TIP60 is recruited to the break by Skb1/PRMT5 which methylate certain residues within the TIP60 protein and activating it. Once the TIP60-NuA4 complex gets there it initiates long range histone removal and further promotes long range resection. This has the effect of biasing repair towards HR pathways. Remarkably, it is known that Ku80 interacts with the break through acetylated H4K16 so TIP60-NuA4 may also be required for loading

of Ku80 [40]. Highlighted is some preliminary evidence on the roles of Mst1/TIP60 and Skb1 in promoting HR mediated repair.

MATERIALS AND METHODS

Strains. The strains that were used as a part of this study are listed in **Table 1**. The construction of the *ura4-his3-ura4* recombination cassette has been published and described in previous literature [41]. The cassette our lab used was slightly modified by cloning the S. *cerevisiae* HO endonuclease restriction site directly upstream of the *his3+* gene. The HO endonuclease sequence is the same as the one described in [42]. Standard yeast genetics have been used to cross the recombinant mutants with the *ura-his-ura* cassette.

TABLE 1. Strains used in this study

Identifier	Genotype	Source
RCP 24	*h+ ura4::ura4-his3-HO-ura4 his3-D1 leu1-32*	Forsburg
RCP 71	*h- rad52::KanMX6-bioneer ura4::ura4-his3-HO-ura4 his3-D1 leu1-32 ade6-M210/216?*	This Study
RCP 228	*h- smt-0 rhp51::kanMX6-bioneer ura4::ura4-his3-ura4 leu1-32 ade6-M216/210 his3-D1*	Forsburg
RCP 178	*h- pku70::KanMX ura4::ura4-his3-HO-ura4 leu1-32 his3-D1 ade6-M210*	This Study
RCP 256	*h+ rad52::KanMX6-bioneer rad51::kanMX6-bioneer ura4::ura4-his3-ura4 leu1-32 his3-D1 ade6-M216/210*	This Study
RCP 258	*h- rad52::KanMX6-bioneer pku70::kanR ura4::ura4-his3-HO-ura4 leu1-32 his3-D1 ade6-M210*	This Study
RCP 275	*h- smt-0 rad51::kanMX6-bioneer pku70::KanR ura4::ura4-his3-ura4 his3-D1 leu1-32 ade6-M216/210*	This Study
RCP 124	*h+ ura4::ura4-his3-HO-ura4 his3-D1 leu1-32/ Plasmid 41*	This Study
RCP 81	*h- rad52::KanMX6-bioneer ura4::ura4-his3-HO-ura4 his3-D1 leu1-32 ade6-M210/216 /Plasmid 41*	This Study
RCP 267	*h- smt-0 rad51::kanMX6-bioneer ura4::ura4-his3-ura4 leu1-32 his3-D1 ade6-M216/210 / plasmid 41*	This Study
RCP 371	*h- pku70::KanMX ura4::ura4-his3-HO-ura4 leu1-32 his3-D1 ade6-M210/ plasmid 41*	This Study
RCP 268	*h+ rad52::KanMX6-bioneer rad51::kanMX6-bioneer ura4::ura4-his3-ura4 leu1-32 his3-D1 ade6-M216/210 /plasmid41*	This Study
RCP 288	*h- rad51::kanMX6-bioneer pku70::kanMX ura4::ura4-his3-ura4 leu1-32 ade6-M216/210 ura4-D18 his3-D1 / plasmid 41*	This Study
RCP377	*h- pku70::KanMX rad52::KanMX-bioneer ura4::ura4-his3-HO-ura4 leu1-32 his3-D1 ade6-M210 / plasmid 41*	This Study

Spontaneous break recombination protocol. The assay was done as follows. Cells were struck out from the -70°C freezer onto EMM-His plates and grow at 32°C for 3-4 days until colonies appear. 10 colonies were resuspended in water, the cells were counted, and released in 4mL of liquid YES at 100 cells/microliter. Incubate tubes at 32°C in the rotator for approximately 48 hr. To determine concentration of cells in the tubes, the counting was done using a hemocytometer and the cells were plated onto EMM-Uracil + Phloxin B at 10^5-10^6 cells per plate. Because ura- cells tend to cannibalize themselves sometimes false positives appear. The addition of Phloxin B makes it easier to identify false positive because it stains ura- cells bright red. Furthermore, using large 150 X 15mm plates particularly when plating at higher density is recommended. YES, controls were plated as well for each colony plated on EMM-Uracil at 10^3 cells per

plate. This control is important to check for cell viability and accuracy in counting. All the plates were incubated at 32°C until colonies appeared. The colonies on both YES and EMM-Uracil plates were counted and their numbers recorded.

Induced break recombination protocol. Cells were struck out onto EMM-Leucine+Thiamine plates from the -70°C freezer and incubate at 32°C for 3-4 days until single colonies appeared. 10 single colonies were then resuspended in water, their cells counted and release in 4 mL liquid EMM Leucine at 100 cells/microliter. The tubes were then incubated at 32°C with rotation for approximately 48 hr. The concentration of the cells in the tubes was determined by using a hemocytometer. The cells were plated onto EMM-Uracil (100 X 15mm plates) at 10^4 cells per plate and on YES at 10^3 cells per plate. All the plates were Incubated at 32° C until colonies appear. Colonies were then counted on both YES and EMM-Uracil plates and their numbers recorded.

PCR analysis. The primers for the polymerase chain reaction (PCR) were, 5'-AGCTACAAATCCCACTGGCT3' and 5'-TGATATTGACGAAACTTTTT-3'. PCR was done using Phusion DNA polymerase and GC buffer at 55°C annealing temperature (34 cycles).

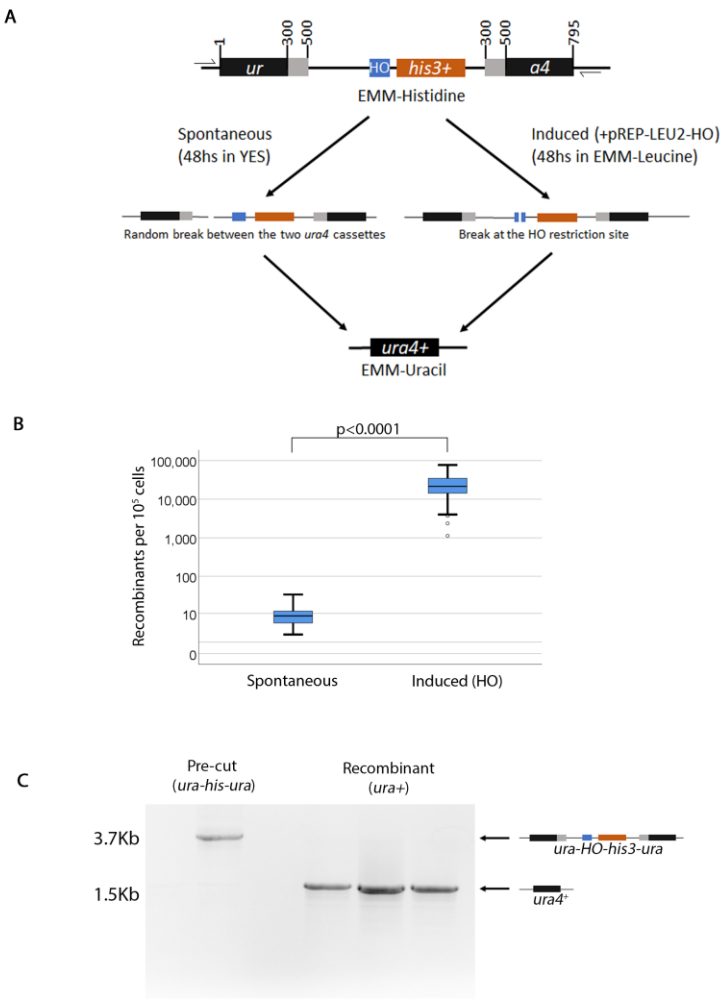

Figure 4. An assay to study spontaneous and induced double strand breaks at regions of non-tandem repeats. A. The *ura-his-ura* assay. In this assay two non-functional *ura4* alleles flank a functional *his3*$^+$ allele. The *ura4* alleles have 200 bps of identical overlapping sequence creating two non-tandem repeats. The *S. cerevisiae* homothallic endonuclease (*HO*) is cloned just upstream of the *his3*$^+$ gene. The *HO* enzyme is on a *LEU2* plasmid under the control of the *nmt1* promoter which can be repressed with thiamine. Spontaneous *ura4*$^+$*his3*$^-$ recombinants are assayed by growing cells in non-selective YES media for 48 hr then plating on selective EMM-Uracil. Induced break recombinants are assayed by growing cells for 48 hr in media without thiamine to de-repress the *HO* endonuclease while maintaining selection for the plasmid (EMM-Leucine). Cells are then plated on EMM-Uracil. All experiments were done at 32°C. **B**. Box plot showing frequency of recombinants for both induced and spontaneous breaks. **C**. PCR across the *ura-his-ura* cassette in both pre and post-recombination strains. Half arrowheads in **A** show approximate positions of primers.

Data analysis. For all assays, the data were adjusted for viability and error in plating using the numbers on the YES plates (# colonies EMM-Uracil / (# colonies on YES/1000). This normalization was also important to control for systematic errors that might have been introduced as different people did the experiments. Descriptive statistics and graphs were generated using SPSS. Independent samples t-test probabilities were computed using the online calculator.

TABLE 2. Descriptive statistics for spontaneous breaks

	N	Mean		Std. Deviation
	Statistic	Statistic	Std. Error	Statistic
WT	30	9.8997	1.2666	6.9375
rad52Δ	35	2.1223	.3730	2.2068
rad51Δ	55	156.1130	17.5819	130.3907
pku70Δ	24	41.3799	5.2449	25.6948
rad52Δ rad51Δ	48	4.1259	.6046	4.1890
rad52Δ pku70Δ	29	6.3050	.8304	3.6196
rad51Δ pku70Δ	29	96.4710	13.5511	72.9750

RESULTS AND DISCUSSION

Several *in vivo* assays have been designed in various model systems as well as human cells to study the genetic requirements for DSB repair (we only reference a few) [8, 43-50]. Such studies have contributed vastly to our understanding of how chromosomal instability arises. The Petreaca laboratory previously briefly described an assay to study chromosomal instability at non-tandem direct repeats arising from spontaneous damage [41]. However, this assay was only briefly characterized and not sufficiently validated. Here, we present an improved assay that can be used to study both random and induced DSBs and describe the protocol for this method. We also provide preliminary characterization of the genetic requirements for repair of these different types of breaks. Finally, we use the assay to characterize the functions of Mst1/TIP60 and Skb1.

TABLE 3. Descriptive statistics for induced breaks

	N	Mean		Std. Deviation
	Statistic	Statistic	Std. Error	Statistic
WT	49	2583.4854	254.6167	1782.3176
rad52Δ	34	99.8696	10.9486	63.8409
rad51Δ	46	35.1195	5.2726	35.7610
pku70Δ	27	4140.3395	543.7249	2825.2775
rad52Δ rad51Δ	39	.4639	.0792	.4944
rad52Δ pku70Δ	35	242.2844	29.2442	173.0116
rad51Δ pku70Δ	28	13.5361	4.6545	24.6295

An assay to study repair at direct repeats. In this assay, two non-functional *ura4* fragments are placed on either side of a functional *his3*$^{+}$ gene (**Fig. 4A**). The *ura4*$^{+}$ fragments contain 200bp of overlapping identical sequence (arrows) creating two non-tandem repeats (referred to from here on as the *ura-his-ura* cassette). Here, we improved on this assay by introducing the *S. cerevisiae* homothallic endonuclease (*HO*) next to the *his3*$^{+}$ gene. The endonuclease restriction site is identical to that described in [42]. Using this assay, we can monitor both random breaks that may arise during DNA replication and HO induced breaks. The HO endonuclease is expressed from the p*REP81X-HO* plasmid [13].

Spontaneous *ura4⁺his3⁻* recombinants arise at an average frequency of approximately 1 in 10^4 cells (**Fig. 4B, Table 2**). As expected, when the break is made by the endonuclease, the rate is much higher (2.5 in 10 colonies) (**Fig. 1B, Table 2**). This assay does not appear to report conversion (e.g. *ura4⁺his3⁺* colonies). PCR analysis of several recombinants with primers flanking the *ura4⁺* ORF showed that the *ura-*

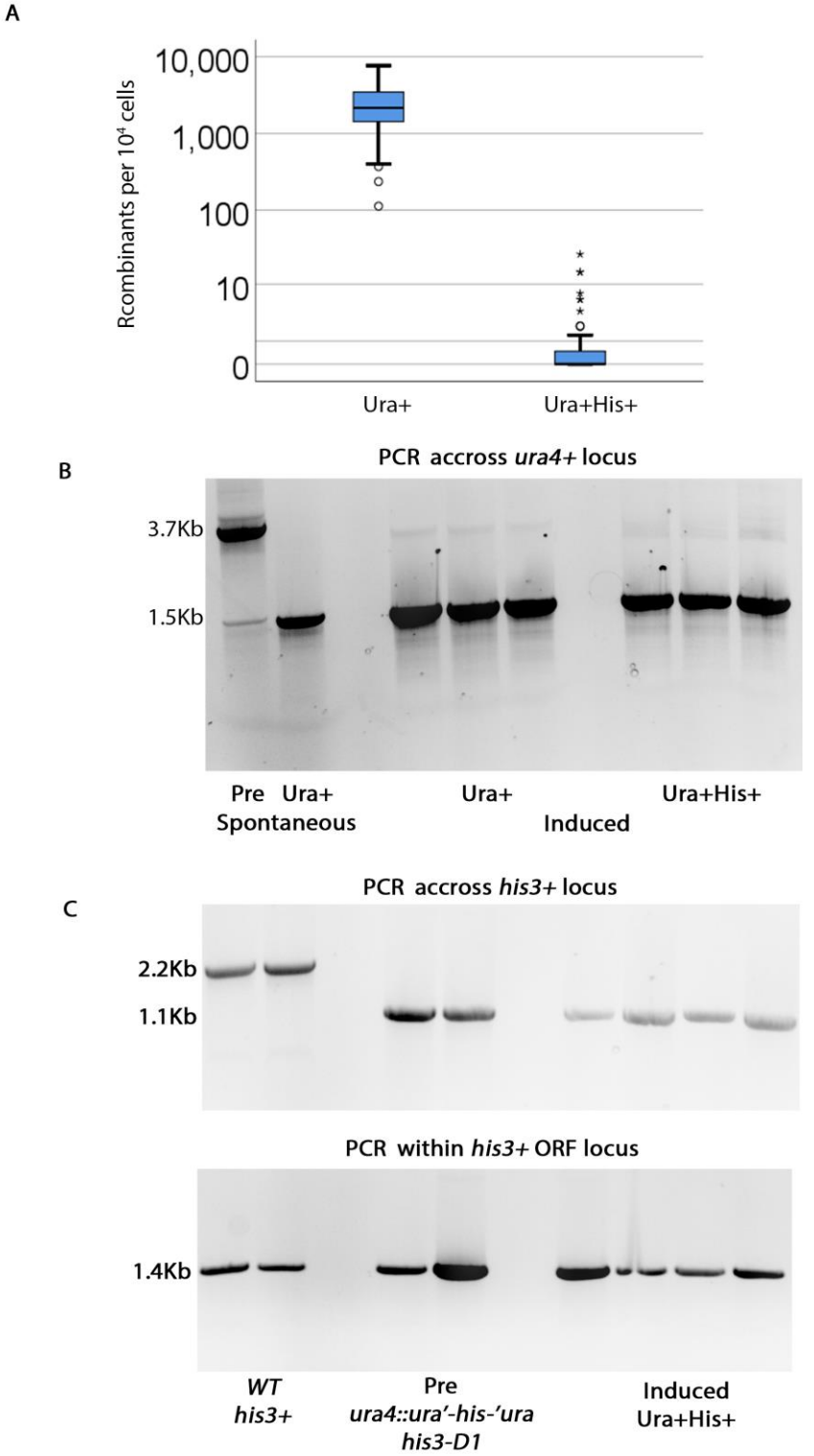

Figure 5. Analysis of Ura+His+ recombinants. A. Box-plot comparing the frequency of Ura+ and Ura+His+ recombinants. Note that the scale is logarithmic and that the frequency of the Ura+His+ is approximately three orders of magnitude lower. **B**. PCR across the *ura4⁺* locus with same primers form Figure 1C. Shown are pre-recombinants (e.g. intact *ura-his-ura*) and post recombinants Ura+ and Ura+His+. Only when the break is induced can Ura+His+ be recovered. **C.** PCR to check the his3+ locus on chromosome 2 in WT (*his3⁺*), Pre (*ura4::ura'-his-'ura, his3-D1*) and induced recombinants Ura+His+. The top gel labeled "PCR across *his3⁺* locus" is PCR with primers upstream and downstream the his3⁺ open reading frame. Note that the WT (*his3⁺*) has a longer fragment than both pre and post recombinants which should be *his3-D1*. The *his3-D1* allele is a deletion of most of the *his3⁺* ORF. This indicates that the *his3-D1* locus has not been converted to *his3⁺*. The bottom gel labeled "PCR within the *his3⁺* ORF" is PCR with primers within the *his3⁺* ORF. Note that all samples produce the same band indicating that they all have an intact *his3⁺* ORF.

his-ura cassette has been converted to *ura4⁺* (**Fig. 4C**). Sporadically, we did find some colonies that were *ura4⁺his3⁺*, which appeared at a much lower rate and only when we induced the break (**Fig. 5**). To understand what these *ura4⁺his3⁺* colonies were, we used PCR to check the size of the locus in the *HO* recombinant colonies (**Fig. 5B**). When primers flanking the *ura4⁺* ORF are used, we found that both the *ura4⁺* and the *ura4⁺his3⁺* are the same size indicating that both are deletion outcomes. We next checked whether the *ura4⁺his3⁺* colonies arose as a result of gene conversion between the *his3⁺* in our assay and the *his3-D1* locus [51]. PCR across the *his3⁺* locus showed that the *his3-D1* deletion is present in both the *ura-his-ura* (pre) and the recombinant *ura4⁺his3⁺* colonies suggesting that the *his3-D1* allele has not been converted to *his3⁺* (WT) (**Fig. 5C**). However, PCR with primers within the ORF *his3⁺* detected the presence of the *his3⁺* ORF in the *ura4⁺his3⁺* strains. We concluded from this PCR analysis and the very low rate of the *ura4⁺his3⁺* recombinants that the *his3⁺* must arise due to some spurious integration of the ORF elsewhere in the genome. Thus, this assay can be used to test primarily deletions.

TABLE 5. Two tailed p-values for independent samples *t-test*. *Induced breaks.*

	WT	***rad52Δ***	***rad51Δ***	***pku70Δ***	***rad52Δ rad51Δ***	***rad52Δ pku70Δ***
rad52Δ	P<0.0001					
rad51Δ	P<0.0001	P<0.0001				
pku70Δ	P=0.0044	P<0.0001	P<0.0001			
rad52Δ rad51Δ	P<0.0001	P<0.0001	P<0.0001	P<0.0001		
rad52Δ pku70Δ	P<0.0001	P=0.0001	P<0.0001	P<0.0001	P<0.0001	
rad51Δ pku70Δ	P<0.0001	P<0.0001	P=0.0064	P<0.0001	P<0.0001	P<0.0001

Analysis of spontaneous breaks. We next carried some preliminary characterization of the genetic requirements for these deletions. We find that *rad52⁺* is required for spontaneous breaks but *rad51⁺* is not (**Fig. 6A**). In fact, *rad51⁺* appears to inhibit deletion outcomes. These results suggest that spontaneous breaks are repaired through some other mechanism that does not involve *rad51⁺*. *rad51⁺* has been previously shown to suppress chromosomal rearrangements in *S. pombe* arising from

TABLE 4. Two tailed p-values for independent samples *t-test*. *Spontaneous breaks*.

	WT	***rad52Δ***	***rad51Δ***	***yku70Δ***	***rad52Δ rad51Δ***	***rad52Δ yku70Δ***
rad52Δ	P<0.0001					
rad51Δ	P<0.0001	P<0.0001				
yku70Δ	P<0.0001	P<0.0001	P<0.0001			
rad52Δ rad51Δ	P<0.0001	P=0.0100	P<0.0001	P<0.0001		
rad52Δ yku70Δ	P=0.0430	P<0.0001	P<0.0001	P<0.0001	P=0.0510	
rad51Δ yku70Δ	P<0.0001	P<0.0001	P=0.0252	P=0.0009	P<0.0001	P<0.0001

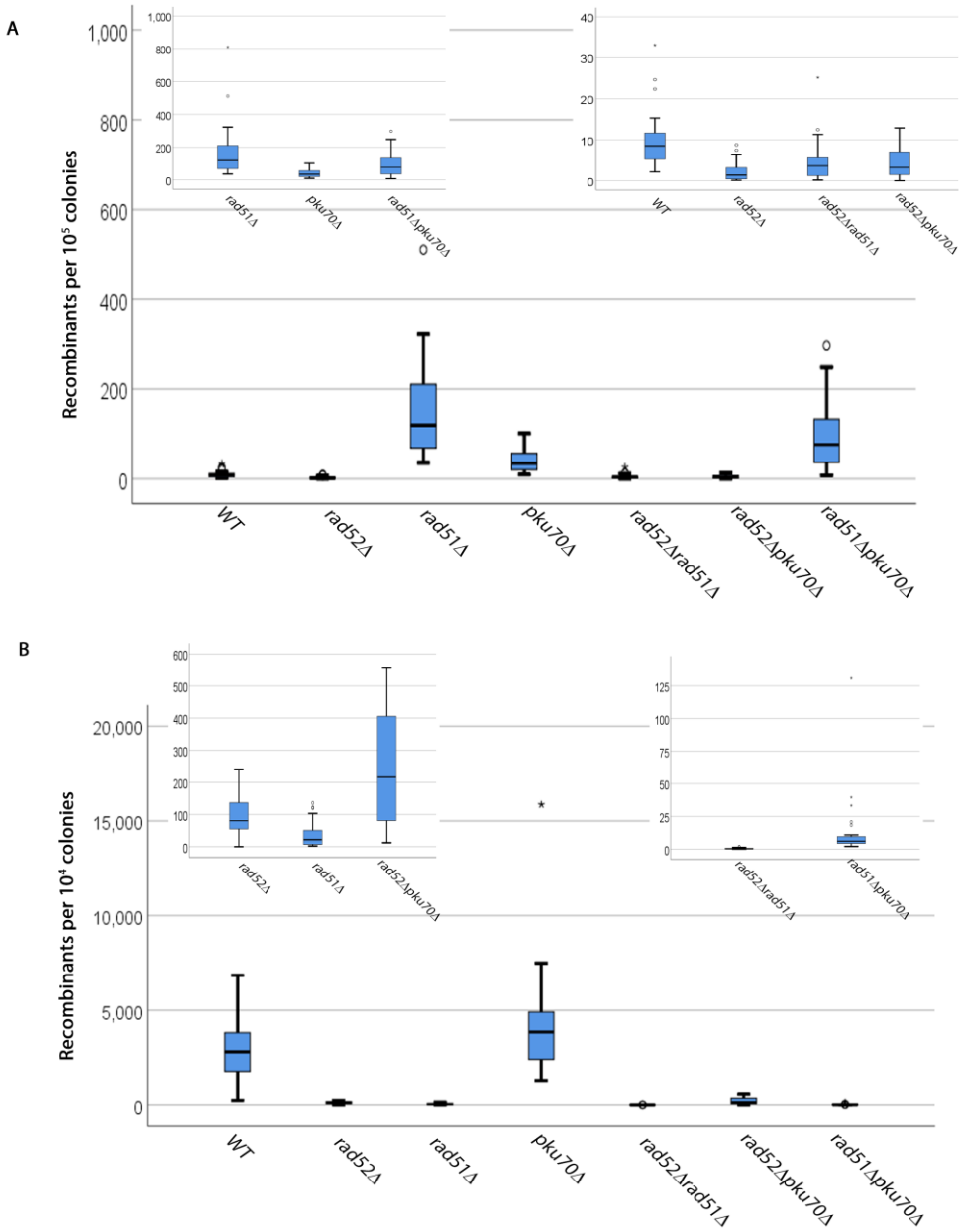

Figure 6. Genetic requirements for spontaneous and induced breaks. A. Box plots showing spontaneous recombination frequency per 10^5 colonies. **B**. Box plots showing induced recombination frequency per 10^4 colonies. For clarity, insets for both A and B are shown for strains with similar recombination frequencies.

improperly repaired spontaneous breaks [49, 52]. Furthermore, mechanisms of repair of spontaneous breaks by single strand annealing that does not rely on *rad51*$^+$ have also been proposed in *S. pombe* [53]. In higher eukaryotes and fission yeast, Rad52 is not essential for all forms of homologous recombination repair [54, 55]. The fact that some repair still occurs in the absence of *rad52*$^+$ indicates that at a low percentage *ura4*$^+$ may be reconstituted by some other form of repair that does not rely on *rad52*$^+$.

Loss of *KU70* (S. pombe *pku70*$^+$*)* also increases recombination outcomes arising from spontaneous breaks indicating that *pku70*$^+$ suppresses these deletions as well. This was not unexpected because non-homologous end joining and *pku70*$^+$ has been previously shown to compete with recombination [13, 56, 57]. The *pku70*$^+$ mechanism of suppression is distinct from *rad51*$^+$ because the difference between the

two mutants is statistically significant (**Table 4**). Deletion of *rad52*$^{+}$ is not epistatic to *rad51Δ* or *pku70Δ* because there is statistical significance between the *rad52Δ* single and the *rad52Δrad51Δ* or *rad52Δ pku70Δ* doubles (**Fig. 4A**, **Table 4**). However, there is no statistical significance between *rad52Δrad51Δ* and *rad52Δpku70Δ* doubles. Taken together, these data suggest that deletions arising from spontaneous breaks are facilitated by *rad52*$^{+}$ while *rad51*$^{+}$ and *pku70*$^{+}$ act in parallel pathways to suppresses this form of non-conservative repair.

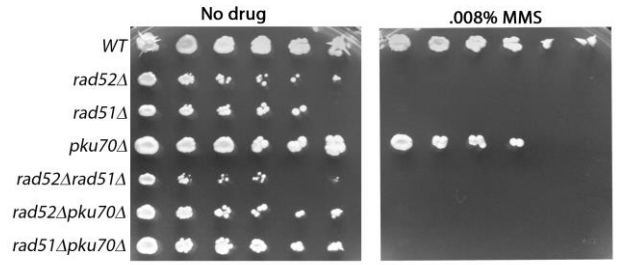

Figure 7. Sensitivity of strains to MMS. Strains of the indicated genotypes were grown in YES at 32°C overnight then 5X serial dilutions were spotted onto YES plates or YES+0.008%MMS. Plates were incubated at 32°C for 4 days. Note that both the *rad52Δ* and *rad51Δ* mutant strains remain sensitive indicating that they did not acquire a suppressor.

Sometimes deletion of *rad52*$^{+}$ in *S. pombe* acquires a suppressor that attenuates sensitivity of the strains to DNA damaging drugs. However, these results are not due to the effect of such a suppressor because all strains used here are still sensitive to methyl-methanosulfonate (MMS) (**Fig.6**).

Analysis of induced breaks. Both *rad52*$^{+}$ and *rad51*$^{+}$ are required for recombinants arising from induced breaks suggesting that induced breaks are likely repaired through a crossover, either intrachromosomal or unequal sister chromatid exchange (**Fig. 6B and Table 5**). Remarkably, *rad52*$^{+}$ and *rad51*$^{+}$ make independent contributions because the difference between the two mutants is statistically significant and the double mutant *rad52 Δ rad51Δ* is sicker than both the single mutants (**Table 5**). As previously shown [56, 57], *pku70*$^{+}$ antagonizes recombination and not unexpectedly we also show that deletion of *pku70* increases recombination outcomes. However, these recombinants are dependent on *rad52*$^{+}$ and *rad51*$^{+}$. These results suggest that the mechanism of repair of induced breaks is distinct from that of spontaneous breaks.

Recombination per 10^4 colonies was observed as low for wild type cells, but even lower for rad52*Δ*. Rad51*Δ* drastically increase in the number of recombinant outcomes, meaning that rad51 must be an antagonist of recombination. Mst1*Δ* and skb1*Δ* are also higher than in wild type and must promote conservative repair. The difference in their amounts maybe attributed to them being in different repair pathways when functioning (**Fig. 8**). When there is a double knock-out of both mst1*Δ* and skb1*Δ* we see that the recombination rate is even higher than each individual knock-out alone giving us further reason to believe that both of these genes promote conservative repair.

Here we describe an assay to study intra-chromosomal deletions arising at regions of non-tandem repeats and provide preliminary data that induced breaks have different genetic requirements than spontaneous breaks. We believe that this assay will be an important tool in the field of DNA damage repair. It is also worth noting that some cell cycle regulators such as CDKN2A are inactivated in cancer cells by deleting the entire gene rather than by point mutations [58, 59]. This shows that these intra-chromosomal deletions could introduce enough genetic change in human cells that may cause cancer. Since there is enough conservation in repair genes between yeast and human cells, this assay could be used to leverage the powerful yeast genetics to identify the mechanisms of these intra-chromosomal deletions.

CONCLUSION

Once a double strand break occurs the cell has to choose a repair pathway. In spontaneous breaks rad52 acts as a switch between conservative HR and non-conservative HR. *rad51*, *skb1* and *mst1* all promote conservative HR while inhibiting SSA. If one of these genes are knocked out than more recombination can occur through the non-conservative pathway (**Fig. 9**). Cells that go through the deletion pathway are more likely to die and prevent cells with significant DNA damage from becoming cancerous. In induced breaks we confirmed previously known data that showed repair will only go through error proof, conservative HR repair.

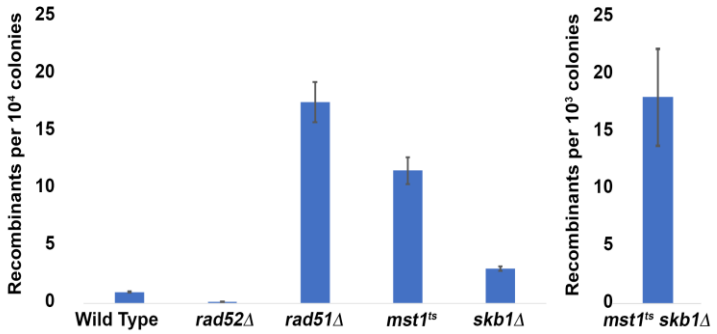

Figure 8. Mst1 and Skb1 promote conservative repair. The assay described in Figure 4A was used to determine these rates.

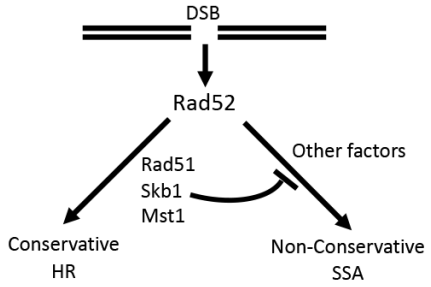

Figure 9. Model for the role of DNA repair genes. Rad51, Skb1 and Mst1 promote conservative HR while inhibiting non-conservative SSA.

REFERENCES

[1] Watson JD, Crick FH. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. J.D. Watson and F.H.C. Crick. Published in Nature, number 4356 April 25, 1953. Nature. 1974;248:765.

[2] Tubbs A, Nussenzweig A. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. Cell. 2017;168:644-56.

[3] Tabancay AP, Jr., Forsburg SL. Eukaryotic DNA replication in a chromatin context. Curr Top Dev Biol. 2006;76:129-84.

[4] Cavalier-Smith T. Origins of the machinery of recombination and sex. Heredity (Edinb). 2002;88:125-41.

[5] Mehta A, Haber JE. Sources of DNA double-strand breaks and models of recombinational DNA repair. Cold Spring Harb Perspect Biol. 2014;6:a016428.

[6] Caldecott KW. Single-strand break repair and genetic disease. Nat Rev Genet. 2008;9:619-31.

[7] Bhargava R, Onyango DO, Stark JM. Regulation of Single-Strand Annealing and its Role in Genome Maintenance. Trends Genet. 2016;32:566-75.

[8] Stark JM, Pierce AJ, Oh J, Pastink A, Jasin M. Genetic steps of mammalian homologous repair with distinct mutagenic consequences. Mol Cell Biol. 2004;24:9305-16.

[9] Biehs R, Steinlage M, Barton O, Juhasz S, Kunzel J, Spies J, et al. DNA Double-Strand Break Resection Occurs during Non-homologous End Joining in G1 but Is Distinct from Resection during Homologous Recombination. Mol Cell. 2017;65:671-84 e5.

[10] Anand RP, Lovett ST, Haber JE. Break-induced DNA replication. Cold Spring Harb Perspect Biol. 2013;5:a010397.

[11] Necsulea A, Popa A, Cooper DN, Stenson PD, Mouchiroud D, Gautier C, et al. Meiotic recombination favors the spreading of deleterious mutations in human populations. Hum Mutat. 2011;32:198-206.
[12] Wang W, Daley JM, Kwon Y, Xue X, Krasner DS, Miller AS, et al. A DNA nick at Ku-blocked double-strand break ends serves as an entry site for exonuclease 1 (Exo1) or Sgs1-Dna2 in long-range DNA end resection. J Biol Chem. 2018;293:17061-9.
[13] Prudden J, Evans JS, Hussey SP, Deans B, O'Neill P, Thacker J, et al. Pathway utilization in response to a site-specific DNA double-strand break in fission yeast. EMBO J. 2003;22:1419-30.
[14] Hanamshet K, Mazina OM, Mazin AV. Reappearance from Obscurity: Mammalian Rad52 in Homologous Recombination. Genes (Basel). 2016;7.
[15] Lisby M, Rothstein R. DNA repair: keeping it together. Curr Biol. 2004;14:R994-6.
[16] Kanaar R, Troelstra C, Swagemakers SM, Essers J, Smit B, Franssen JH, et al. Human and mouse homologs of the Saccharomyces cerevisiae RAD54 DNA repair gene: evidence for functional conservation. Curr Biol. 1996;6:828-38.
[17] Dillingham MS, Kowalczykowski SC. RecBCD enzyme and the repair of double-stranded DNA breaks. Microbiol Mol Biol Rev. 2008;72:642-71, Table of Contents.
[18] Rozhok AI, DeGregori J. The evolution of lifespan and age-dependent cancer risk. Trends Cancer. 2016;2:552-60.
[19] DeGregori J. Challenging the axiom: does the occurrence of oncogenic mutations truly limit cancer development with age? Oncogene. 2013;32:1869-75.
[20] Bieging KT, Mello SS, Attardi LD. Unravelling mechanisms of p53-mediated tumour suppression. Nat Rev Cancer. 2014;14:359-70.
[21] Caspari T, Carr AM. Checkpoints: how to flag up double-strand breaks. Curr Biol. 2002;12:R105-7.
[22] Cicenas J, Kvederaviciute K, Meskinyte I, Meskinyte-Kausiliene E, Skeberdyte A, Cicenas J. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 Mutations in Pancreatic Cancer. Cancers (Basel). 2017;9.
[23] Cicenas J, Tamosaitis L, Kvederaviciute K, Tarvydas R, Staniute G, Kalyan K, et al. KRAS, NRAS and BRAF mutations in colorectal cancer and melanoma. Med Oncol. 2017;34:26.
[24] Agarwal P, Miller KM. The nucleosome: orchestrating DNA damage signaling and repair within chromatin. Biochem Cell Biol. 2016;94:381-95.
[25] Jeggo PA, Downs JA. Roles of chromatin remodellers in DNA double strand break repair. Exp Cell Res. 2014;329:69-77.
[26] House NC, Koch MR, Freudenreich CH. Chromatin modifications and DNA repair: beyond double-strand breaks. Front Genet. 2014;5:296.
[27] Yang XJ. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. Nucleic Acids Res. 2004;32:959-76.
[28] Avvakumov N, Cote J. The MYST family of histone acetyltransferases and their intimate links to cancer. Oncogene. 2007;26:5395-407.
[29] Avvakumov N, Cote J. Functions of myst family histone acetyltransferases and their link to disease. Subcell Biochem. 2007;41:295-317.
[30] Gomez EB, Nugent RL, Laria S, Forsburg SL. Schizosaccharomyces pombe histone acetyltransferase Mst1 (KAT5) is an essential protein required for damage response and chromosome segregation. Genetics. 2008;179:757-71.
[31] Allard S, Utley RT, Savard J, Clarke A, Grant P, Brandl CJ, et al. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. Embo J. 1999;18:5108-19.
[32] Bird AW, Yu DY, Pray-Grant MG, Qiu Q, Harmon KE, Megee PC, et al. Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. Nature. 2002;419:411-5.
[33] Gomez EB, Espinosa JM, Forsburg SL. Schizosaccharomyces pombe mst2+ encodes a MYST family histone acetyltransferase that negatively regulates telomere silencing. Mol Cell Biol. 2005;25:8887-903.

[34] Nugent RL, Johnsson A, Fleharty B, Gogol M, Xue-Franzen Y, Seidel C, et al. Expression profiling of S. pombe acetyltransferase mutants identifies redundant pathways of gene regulation. BMC Genomics. 2010;11:59.
[35] Gilbreth M, Yang P, Wang D, Frost J, Polverino A, Cobb MH, et al. The highly conserved skb1 gene encodes a protein that interacts with Shk1, a fission yeast Ste20/PAK homolog. Proc Natl Acad Sci U S A. 1996;93:13802-7.
[36] Stopa N, Krebs JE, Shechter D. The PRMT5 arginine methyltransferase: many roles in development, cancer and beyond. Cell Mol Life Sci. 2015;72:2041-59.
[37] Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. Annu Rev Genet. 2011;45:247-71.
[38] Clerici M, Baldo V, Mantiero D, Lottersberger F, Lucchini G, Longhese MP. A Tel1/MRX-dependent checkpoint inhibits the metaphase-to-anaphase transition after UV irradiation in the absence of Mec1. Mol Cell Biol. 2004;24:10126-44.
[39] Foster SS, Balestrini A, Petrini JH. Functional interplay of the Mre11 nuclease and Ku in the response to replication-associated DNA damage. Mol Cell Biol. 2011;31:4379-89.
[40] Miller KM, Tjeertes JV, Coates J, Legube G, Polo SE, Britton S, et al. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol. 2010;17:1144-51.
[41] Li PC, Petreaca RC, Jensen A, Yuan JP, Green MD, Forsburg SL. Replication fork stability is essential for the maintenance of centromere integrity in the absence of heterochromatin. Cell Rep. 2013;3:638-45.
[42] Du LL, Nakamura TM, Moser BA, Russell P. Retention but not recruitment of Crb2 at double-strand breaks requires Rad1 and Rad3 complexes. Mol Cell Biol. 2003;23:6150-8.
[43] Fortunato EA, Osman F, Subramani S. Analysis of spontaneous and double-strand break-induced recombination in rad mutants of S. pombe. Mutat Res. 1996;364:14-60.
[44] Cullen JK, Hussey SP, Walker C, Prudden J, Wee BY, Dave A, et al. Break-induced loss of heterozygosity in fission yeast: dual roles for homologous recombination in promoting translocations and preventing de novo telomere addition. Mol Cell Biol. 2007;27:7745-57.
[45] Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev. 1999;13:2633-8.
[46] Sugawara N, Haber JE. Repair of DNA double strand breaks: in vivo biochemistry. Methods Enzymol. 2006;408:416-29.
[47] Gonzalez-Barrera S, Cortes-Ledesma F, Wellinger RE, Aguilera A. Equal sister chromatid exchange is a major mechanism of double-strand break repair in yeast. Mol Cell. 2003;11:1661-71.
[48] Liddell L, Manthey G, Pannunzio N, Bailis A. Quantitation and analysis of the formation of HO-endonuclease stimulated chromosomal translocations by single-strand annealing in Saccharomyces cerevisiae. J Vis Exp. 2011.
[49] McDonald JP, Rothstein R. Unrepaired heteroduplex DNA in Saccharomyces cerevisiae is decreased in RAD1 RAD52-independent recombination. Genetics. 1994;137:393-405.
[50] Osman F, Fortunato EA, Subramani S. Double-strand break-induced mitotic intrachromosomal recombination in the fission yeast Schizosaccharomyces pombe. Genetics. 1996;142:341-57.
[51] Burke JD, Gould KL. Molecular cloning and characterization of the Schizosaccharomyces pombe his3 gene for use as a selectable marker. Mol Gen Genet. 1994;242:169-76.
[52] Nakamura K, Okamoto A, Katou Y, Yadani C, Shitanda T, Kaweeteerawat C, et al. Rad51 suppresses gross chromosomal rearrangement at centromere in Schizosaccharomyces pombe. EMBO J. 2008;27:3036-46.
[53] Onaka AT, Toyofuku N, Inoue T, Okita AK, Sagawa M, Su J, et al. Rad51 and Rad54 promote noncrossover recombination between centromere repeats on the same chromatid to prevent isochromosome formation. Nucleic Acids Res. 2016;44:10744-57.

[54] Osman F, Adriance M, McCready S. The genetic control of spontaneous and UV-induced mitotic intrachromosomal recombination in the fission yeast Schizosaccharomyces pombe. Curr Genet. 2000;38:113-25.
[55] Yamaguchi-Iwai Y, Sonoda E, Buerstedde JM, Bezzubova O, Morrison C, Takata M, et al. Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in RAD52. Mol Cell Biol. 1998;18:6430-5.
[56] Tomita K, Matsuura A, Caspari T, Carr AM, Akamatsu Y, Iwasaki H, et al. Competition between the Rad50 complex and the Ku heterodimer reveals a role for Exo1 in processing double-strand breaks but not telomeres. Mol Cell Biol. 2003;23:5186-97.
[57] Ferreira MG, Cooper JP. Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. Genes Dev. 2004;18:2249-54.
[58] Florl AR, Schulz WA. Peculiar structure and location of 9p21 homozygous deletion breakpoints in human cancer cells. Genes Chromosomes Cancer. 2003;37:141-8.
[59] Frazao L, do Carmo Martins M, Nunes VM, Pimentel J, Faria C, Miguens J, et al. BRAF V600E mutation and 9p21: CDKN2A/B and MTAP co-deletions - Markers in the clinical stratification of pediatric gliomas. BMC Cancer. 2018;18:1259.